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THE
BOTANICAL GAZETTE

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CELL AND NUCLEAR DIVISION IN CLOSTERIUM

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(WITH PLATES XXII AND XXIII AND ONE FIGURE)

Historical

The first figures showing division in desmids were those of *Cosmarium* by EHRENBERG (**11**). Those drawings, while not entirely accurate, indicate clearly that he saw the two new daughter half-cells being interpolated between the old ones. Each half of the parent cell was evidently considered by him as an individual, since his genus description states that the individuals are arranged in the colonies "in chains of two or four."

NÄGELI (**33**), RALFS (**35**), and FOCKE (**17**) observed cell division in the desmids and gave fairly complete accounts of the process. DEBARY (**9**) did not describe division in detail, but mentions the fact that the newly formed transverse wall of *Closterium* and *Penium* is flat, and that the new end grows out as a cone-shaped structure. As the chromatophore is divided into two parts, some of the older observers, as EHRENBERG, regarded the mature plant as a chain of two cells, but DEBARY was clear on this point and recognized the desmid as a single cell with a nucleus between the halves. Nothing was observed by any of these investigators as to the conduct of the nucleus during division, as the importance of that organ of the cell was not yet fully recognized.

It is to ALFRED FISCHER (**14**) that we owe our first knowledge of the details of the process as it occurs in this genus. FISCHER found the cross-wall formed in essentially the same manner that STRASBURGER had described for *Spirogyra*. It appears soon after

nuclear division, at the middle of the cell at the point where the old nucleus lay, and cuts across the cell at right angles to its long axis. It is only after the complete isolation of the two halves of the old cell that the new ends of each *Closterium* grow out again to restore the symmetry of the chromatophore and cell outline in each individual. In each new end there lies a dense accumulation of cytoplasm, and in this is the daughter nucleus. FISCHER saw that immediately after nuclear division the daughter nuclei move back from the position occupied by the mother nucleus. They migrate out at right angles to the long axis of the cell, and move back from the equator of the cell to their new position in the forming furrow of the chromatophore. FISCHER watched this process eight times in living specimens of *C. Delpontii*, and found that the migration is a very rapid one and may occur along either the convex or concave side. In *C. Delpontii* this migration had frequently been completed before the two cells had pulled apart, and in *C. moniliferum* the nucleus frequently came to rest in the furrow in the chromatophore before that body had been entirely divided, the latter part of the process seeming to be completed under its direction. In the passage backward around the chromatophore the nucleus seems to press that body to one side to make room for it to pass. The granular protoplasm that had gathered at the middle of the mother cell forms the tip of each new half, and apparently assists as MOLL'S embryonic substance in the very rapid growth of the new membrane. This new half rounds out, the protoplasm streams into it, and the plant soon takes on a symmetrical shape. The new end vacuole appears in the granular tip, which even in the adult remains without chlorophyll. In *C. Delpontii* the entire process, beginning at about midnight, was completed and the two halves had become symmetrical in about five hours. FISCHER points out the further interesting fact that in the young *Closterium* the chromatophore in either end grows so that the two halves become pressed on each other, forming an apparent, but of course not real, fusion, as the two halves of the chromatophore remain separate throughout the life of the individual. He also noted that these observations on cell division give the reason for the conformity of the ridges of the chromatophore on either side of the nucleus.

FISCHER (**15**) in a later paper gave further details as to the origin in the new half of the end vacuoles and its crystals. He believed that the latter formed in the cytoplasm and later migrated into the vacuole.

Some of FISCHER'S observations on the method in which the cell wall divides were criticized by both GAY (**18**) and HAUPTFLEISCH (**23**). GAY'S paper I have not seen, but according to HAUPTFLEISCH, who reviews it, he points out that FISCHER'S results hold only for wall division in those varieties with thick walls on which were longitudinal and cross-markings. HAUPTFLEISCH showed further that in those forms without girdle rings, such as *C. Ehrenbergii* and *C. moniliferum*, the isolation of the daughter cells is accomplished by a simple splitting of the cross-wall.

Considerable work has been done by LÜTKEMÜLLER (**26**) on the formation of the cell wall during the division process, but as little of his work has direct bearing on division in the species discussed in this paper, it will not be reviewed. Based on this work, LÜTKEMÜLLER has attempted to formulate a consistent scheme of phylogeny for the desmid group. He regards them as degenerate filamentous conjugates. This view he shares with WEST (**46**), who, as a result of studies on variation in desmids, had published an almost identical theory of their phylogeny three years previously.

The very peculiar nucleus of the desmids was evidently a puzzle to the early observers. DEBARY (**9**) does not attempt to describe it, but BRAUN (**5**) has the following to say: "The nucleus, with its colorless mucilaginous envelope, is maintained in the center of the spindle-shaped cell by the green lamellae of contents, arranged radiantly around the long axis of cell, which lamellae are interrupted by it in the middle of the cell." From this it will be seen that he evidently regarded the central granular mass as the nucleus, while the nuclear reticulum was the "mucilaginous envelope."

I have described (**27**) the resting cell of *Closterium* with special reference to the chromatophore and pyrenoids, but have given also briefly some idea of the peculiar structure of the nuclei in *C. Ehrenbergii* and *C. moniliferum*. The particular feature to which I called attention was the great accumulation of stainable material

at the center of the nucleus, which seemed to take the place of the nucleus or nucleoli of other plants.

All of this work on *Closterium*, with the exception of my investigation, it will be noted has been done either on the living cells or on fixed and stained whole mounts. The only members of the Conjugatae that have been studied with reference to cell and nuclear division in sectioned material are *Spirogyra* and *Zygnema*. Many investigators, on account of the ease with which the nuclear process can be followed in the filament, have preferred to use fixed and stained but unsectioned filaments, but others have resorted to sectioned material as the best means for seeing the finer details of the nuclear phenomena.

In *Spirogyra*, as is well known, the nucleus contains one or more nucleoli that are proportionally exceedingly large. On account of the great mass of nucleolar material, it has been generally accepted that the network outside the nucleoli either was very poor in chromatin or was entirely free from it, the entire chromatic material being concentrated in the central bodies which have been termed the chromatin nucleoli. A dispute has centered around the origin of the chromosomes; whether they came from the reticulum around the nucleolus or from the nucleolus itself. From the time when STRASBURGER (**37**) first investigated divisions in *Spirogyra* to the latest paper by KARSTEN (**25**) on divisions in the young plant as it comes out of the zygospore, the opinions of the investigators as to the origin of the chromosomes have been divided. Altogether over twenty papers have appeared on the subject, making the nuclear divisions of *Spirogyra* the most thoroughly studied of those of any algal form.

In STRASBURGER'S original contribution, which appeared in *Zellbildung und Zelltheilung* (1875), he claimed that the nucleolus or nucleoli by first falling into granules, which arrange themselves at the middle of the spindle, form the plate. This view he modified in 1882 (**37**) when he returned again to the study of *Spirogyra*, then arriving at the conclusion that in the species he was studying (*S. majuscula*) there was a nuclear cavity, the ends of the chromatin loops being at the poles of the spindle. This structure forms the equatorial plate, the nucleolus in the meantime having disappeared

and its substance having been used up probably in the formation of this spireme. After metaphase the chromosomes form two new spiremes, one at either pole, in the midst of which the nucleoli reappear. In 1884, in *Spirogyra nitida*, STRASBURGER (**38**) observed that the spireme appeared close to the nucleolus, which as a result seemed to take on a granular corroded appearance. In his paper in 1888 (**39**) he finds that in *Spirogyra polytaeniata* a typical spireme, gradually becoming denser, is formed in the nuclear reticulum, while the nucleolus disappears about the end of its formation. Twelve chromosomes are formed, which during the telophase form new spiremes and become reticulated. In other words, at this time in this species of *Spirogyra*, STRASBURGER believed that the nucleoli do not behave very differently in mitosis from those of higher plants.

FLEMMING (**16**) considered the spireme to be formed partly from the material in the nucleolus which became arranged in it. The achromatic part of the reticulum is utilized in the formation of the spindle. TANGL (**41**) claimed that the equatorial plate arose directly from the nucleolus, while CARNOY (**6**) believed that all the chromatic material in the nucleus was collected in the nucleolus, forming there a still smaller body which he proposed calling the nucleo-nucleolus. The nuclear cavity surrounding this body was empty. MEUNIER (**30**) confirmed these observations, finding a spireme formed inside the nucleolus in the early prophases. In the reconstruction stages the achromatic basis of the nucleolus is established first, and then on this the chromatic substance is deposited.

ZACHARIAS (**48**), as a result of microchemical tests, considered the nucleolus as pure plastin and similar to that of the higher plants.

DEGAGNY (**7**) in 1890 believed that while there was chromatin both in the nucleolus and in the extra-nucleolar part of the nucleus, it was the nucleolus alone that formed the plate. In later papers, however, he changed this opinion, and derived the plate from the spireme which comes to envelop the nucleolus closely and seems to absorb the substance from it.

Both MOLL (**32**) and MITZKEWITCH (**31**), applying modern

cytological technic and using microtome sections, arrived at the conclusion that practically all the chromatin was centered in the nucleolus. MOLL found the chromatin coming out of the nucleolus, leaving the latter empty. This chromatin then formed a spireme which cut up into 12 chromosomes. MITZKEWITCH found in the nucleolus two constituents, chromatin and linin. The chromatin appeared in the early prophases of division in the form of a definite number (24) of bodies imbedded in a mass of more lightly staining linin. This mass occupies the position formerly held by the nucleolus, and apparently seems to have resulted from its transformation.

VAN WISSELINGH (**42, 43, 44**), by a process of progressive digestion of the various parts of the nucleus by 40 per cent chromic acid, came to the conclusion that the nucleolus contains chromatin. He also found chromatin in the surrounding reticulum. He ascribed the origin of only two of the chromosomes to the nucleoli; if there are two nucleoli one chromosome comes from each; the other ten arise from the nuclear reticulum.

BERGHS (**2**) has recently devoted a very long paper to a detailed account of the process as he finds it in a *Spirogyra* which he believes to be *S. nitida*. He finds all of the 12 chromosomes arising from the nucleolus, the reticulum surrounding it being entirely free from chromatin. After the chromosomes are formed, there still remains in the nucleolus a second substance which stains less deeply. This substance divides transversely into two groups of pieces which move to the poles with the chromosomes. The nucleus is reconstructed out of these two substances, which, after undergoing vacuolization, are condensed in the nucleolus.

KARSTEN (**25**) finds in the first division of the fusion nucleus in the zygospore of *Spirogyra jugalis* that 14 tetrads arise from the nucleolus, which lies surrounded by light plasma containing two or more weakly staining chromatin spheres, apparently comparable to the extruded nucleoli of other plants. He has followed the development of these chromosomes and finds them appearing gradually in the dark stained nucleolar mass. This stage he believes to be comparable to synapsis in the higher plants.

Miss MERRIMAN (**29**) finds in *Zygnema* a central body which

apparently gives rise to part of the chromosomes, the remainder coming from extra-nucleolar granules. In these opinions it will be seen that she agrees with the results of VAN WISSELINGH (**43**) on *Spirogyra*. At no time did she find a spireme formed. In the telophase, part of the chromosomes, the number apparently not having been determined, fuse to form this central body (nucleolus), while the remaining ones are distributed throughout the nuclear cavity as granules.

ESCOYEZ (**12**) has also recently studied *Zygnema* in GRÉGOIRE'S laboratory, and has arrived at quite different conclusions from those of Miss MERRIMAN. While the nucleolus is very large, the nuclear reticulum furnishes all the chromosomes. He does not deny the fact that the nucleolus may supply some of the chromatic material, but is certain that the morphological chromosomes do not come from it. He finds the chromosomes to arise, not by the fusion of granules as Miss MERRIMAN describes, but in the form of slender rods. In the telophase, the nucleolus is not formed by the union of the chromosomes at the center, but appears to arise independent of the chromatic reticulum.

The spindle in *Spirogyra* is believed by STRASBURGER (**39**), MITZKEWITCH (**31**), VAN WISSELINGH (**44**), and BERGHS (**2**) to be purely of cytoplasmic origin, while MEUNIER (**30**) thinks it to be partly so. On the other hand, FLEMMING (**16**) believed it to be derived from the nucleus itself. Miss MERRIMAN (**29**) seems to favor the view that the spindle arises intranuclearly in *Zygnema*. ESCOYEZ (**12**) believes that in *Zygnema* it is formed from the cytoplasm, although he has not followed the process in detail.

The very peculiar bodies, nucleolus or some structure corresponding to it, that are present in the resting nucleus of *Closterium*, I have described in my previous paper (**27**). On account of the importance of the question as to the behavior of these bodies in nuclear division, especially with reference to the theories of inheritance, which makes the chromatin or the chromosomes the special idioplasm, it will be well to notice some of the recent opinions relating to the rôle of the nucleolus in the formation of the chromosomes, especially in the higher plants.

WAGER (**45**) has recently quite thoroughly reviewed the litera-

ture on that subject, and has expressed some opinions of his own derived partly from the facts as they have been presented by various observers and partly as a result of his work on nuclear division in the root tip of *Phaseolus*. He arrives at the conclusion that the nucleolus is not an independent organ of the cell, but only a part of the nuclear thread in which a considerable portion of the chromatin may be stored, and from which it may be withdrawn again at the time of chromosome formation. He agrees with DIXON (**10**), therefore, that if the chromatin is the bearer of the hereditary qualities, not only the chromatin granules but also the nucleolus must be taken into account in any theories on the subject.

In a number of other plants outside of the Conjugatae the nucleolus has been reported as containing the larger part or all of the stainable material in the nucleus. GOLENKIN (**19**) finds in *Sphaeroplea* the nucleolus breaking up directly into chromosomes. WOLFE (**47**) describes all the chromatin in the *Nemalion* nucleus as being stored in the nucleolus. BEER (**1**) says that the nucleolus of the cells of the *Riccia* thallus contain practically all the chromatin. He was able also at times to distinguish in it a composite structure, as though it were composed of granules. He believes that the spireme thread becomes thickened by the material from these granules. He also believes that similar conditions are found in one of the mosses (*Funaria*).

ESCOYEZ (**12**) states that the nucleolus in *Stypocaulon* appears to contain the larger part of the chromatin, as the reticulum around it stains very lightly in the iron-alum-hematoxylin. The chromosomes are formed from the reticulum, however, and in the telophase the chromosomes form again a typical reticulum, the nucleolus not being formed by their fusion.

STRASBURGER (**40**), as a result of the fact that he finds in *Marsilia* practically all the stainable material collected in the nucleolus, draws the conclusion that the linin and not the chromatin may be the bearer of the hereditary qualities, while the division of the chromatin is only a device to equalize the food supply of the cells.

The very discrepant accounts of *Spirogyra* given by different investigators, and the very close relationship of *Closterium* to it, make a study of the latter very desirable, especially in view of the

very peculiar structure of the nucleolus as I have previously described it (**27**). If the chromosomes fuse together in the telophase of the divisions of the Conjugatae to form a large central body or bodies, this ought to be a favorable place to find them, for this mass is certainly a composite one.

This work was begun at the University of Wisconsin under the direction of Professor R. A. HARPER, but was completed after leaving his laboratory. I am since greatly indebted to him, however, for his critical reading of the manuscript of this paper and for his suggested changes.

Methods

I have already described in my previous paper (**27**) my method for fixing, imbedding, and sectioning *Closterium*, and will only refer briefly to it here. As had happened in the two preceding years, the lily tanks in the botanical greenhouse at the University of Wisconsin were rapidly becoming covered with *Closterium* on the bottom and sides in the early part of May 1909, the organisms occurring in such abundance that they could be obtained in quantity and under favorable conditions for the study of their asexual reproduction. They have regularly disappeared from the tanks during the winter, whether due to the water becoming cold or from their going into the resting condition could not be determined, but they have just as regularly begun to appear again in quantity about the middle of April. The first series of fixations was made the night of May 2, beginning at 11 P.M. and continuing hourly from that time until 5 A.M. Four other series were fixed during the month of May. It was found that the early stages of chromatophore and nuclear division occurred more frequently early in the evening, even as early as 9 P.M., so the major part of my work has been done on material obtained from that time until midnight. The time of the maximum number of divisions is undoubtedly dependent on the character of the preceding day, whether cloudy or bright, determining the amount of starch stored, and more especially on the temperature of the water, although no accurate observations were made on these factors.

The physiological condition of the individual plants also determines their ability to divide, the particular condition required

being apparently a chromatophore filled with large quantities of starch. The difference in external appearance between those cells which are dividing and those not dividing is very striking; the individuals whose pyrenoids only showed a thin shell of starch and in whose pale green chromatophore there was practically no stroma starch were never found dividing, while those whose pyrenoids were surrounded by thick pieces of starch and whose dark-green chromatophore contained an abundance of free starch were the ones found in division.

Fixation was in Flemming's weaker solution, half-strength, although other fluids were used. This solution caused some shrinkage, but as this was principally in the ends of the chromatophore, it did not affect the phenomena of the nuclear and cell division that were occurring principally at the middle of the cell. When the organisms had been brought into the paraffin in which it was desired to cut them, the previous changes of alcohol, paraffin, etc., having been made by pipetting off the liquid above the *Closterium* lying at the bottom of the vial, the vial containing them was held in ice water for a few minutes. The glass could then be broken away and the layer of paraffin containing the plants sectioned. Staining was largely with the triple stain, although iron-hematoxylin was used to a limited extent. After fixation in the Flemming solution all the nuclear and cytoplasmic structures take differential staining very easily.

In addition to the sectioned material, whole amounts were stained with iron-alum-hematoxylin, gradually transferred to glycerin from the water, and mounted in glycerin.

The same species, which I consider to be *Closterium Ehrenbergii* and *Closterium moniliferum*, whose vegetative cells were described in my previous paper (**27**), were found dividing. In my first series of fixations (May 2) both of these were very abundant, but in all my later attempts I succeeded in getting only *C. Ehrenbergii* in division in quantity, the *C. moniliferum* having in the meantime largely disappeared. As my later fixations were the richer in divisions and were the ones upon which I have depended for the larger part of my work, it was found possible to work out the process in all detail in sections in *C. moniliferum*, although I was able

to get a fairly complete series of stages of division in my whole mounts. Enough division figures were found in the sections, however, to assure me that the process was practically the same in the two species.

External appearance of the division process

FISCHER (**14**) has given a fairly complete description of the process of division as it can be seen both in the living specimens and in stained whole mounts, but in order to understand the structures and phenomena that are found in the sections it was necessary to restudy the whole process in detail, according to FISCHER'S method. It will be remembered, too, that owing to the density of the chromatophore and the granular nature of the cytoplasm, FISCHER was able only to guess at the nuclear changes that were occurring simultaneously with those seen in the cytoplasm. Further, it is practically impossible with whole mounts to discover the details of the method by which the new cross-wall is put across the old cell.

Many species show parts of several generations in their cell walls. LÜTKEMÜLLER (**26**) has made a careful study of a number of these species and genera. In both *C. Ehrenbergii* and *C. moniliferum*, however, the process is a very simple one, and the wall, which is very thin and without markings, does not permit the distinction of the parts belonging to different cell generations.

The first external appearance of division in an individual is a pinching in of the chromatophore about a third of the distance from the middle to the tip (figs. 1–9). As previously noted, this occurs only in individuals that have their dark green chromatophores well filled with starch. This pinching in affects at first only the ridges of the chromatophore and occurs inside the plasma membrane, which becomes pulled away from the chromatophore. The mechanics of this process is difficult to understand. It is plain that the division of the chromatophore is entirely distinct from that of the cell. It has the appearance of being constricted as by a rubber band around it at this point. The division of the chromatopore in *Spirogyra* and other Conjugatae is described as due to the constriction of the entire cell. The chromatophore here would

seem to divide just as the entire cell divides in animals like the amoeba, but the constriction in these animals, it must be noted, is a constriction of the plasma membrane. In the present case it may be due to the constriction of a membrane forming the outer layer of the chromatophore, which may perhaps be regarded as similar to a thin tonoplast bounding a vacuole. Progressive cleavage such as HARPER (**21**) has found in the sporangia of the fungi and slime molds, in which the mass of protoplasm is cut up by irregular cleavage furrows from the surface and from the interior, of course corresponds to the cell division and not to the division of the chromatophore in *Closterium*. The furrows in the sporangia are scattered throughout the cleaving mass, being perhaps due to an extrusion of water from its surface and interior, while in *Closterium* there is only one furrow, and this is localized in a definite zone at the middle of the cell. No nuclear changes are visible at this time, and any theories that connect nuclear division directly with cytoplasmic division, such as those of HEIDENHAIN and KOSTANECKI, would seem to break down in the present case.

The nucleus, in the meantime, has apparently undergone no change that is visible externally (figs. 1, 9), the granular mass at the center being still present. The nucleus is in the process of spireme and chromosome formation during the time the chromatophore is dividing, although this process is not visible externally. Soon, however, some individuals (fig. 2) show the chromosomes in the equatorial plate stage on a cylindrical spindle whose ends are hidden by the projecting chromatophores. The nucleus now apparently disappears (fig. 3); the chromosomes, being drawn back to the ends of the spindle, are under the ends of the chromatophore and cannot be seen. Across the middle of the cell there is now (fig. 3) a very conspicuous broad band of granular matter, in the middle of which the new wall is put across. The two nuclei, having been reconstructed, can then be seen moving out to the surface of the chromatophore and making their way back (fig. 4), immediately under the plasma membrane, to the new position they are to occupy permanently in the new cell at the middle of each chromatophore. FISCHER (**14**) states that these migrations

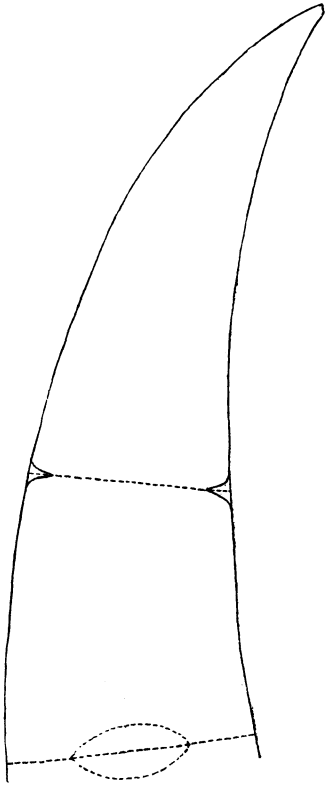
occur on both the concave and convex sides, but I have found them in these species to be practically all on the convex side.

The movement of the nuclei is apparently amoeboid, comparable to that of the male nucleus of the flowering plants in its progress toward the egg nucleus in the embryo sac. In the present case, however, it seems conceivable that the pushing outward of material into the new end of either daughter *Closterium*, combined with the change of shape which that end undergoes as soon as the new wall is formed, may assist to some extent in the movement of the nuclei. It will not explain it entirely, however, and we are forced to assume some stimulus that induces the nucleus to move to its new position. As long as the nucleus is at the end or along the side of the chromatophore, the two sides of the nucleus are exposed unequally to the food supply, and while the active streaming movements in the peripheral granular layer would partly equalize the deficiency, the side that is next the chromatophore is in touch with the soluble foods that are available there in greater quantity than at the side next the granular layer. The only way in which the nucleus can supply itself equally with food over its entire exterior surface is to imbed itself near the center of the chromatophore, and this is the position of course which it finally assumes. Other stimuli may of course also be in play.

Soon after the new cross-wall is put in at the middle of the desmid, the new end begins to round out, but the two organisms hang together for quite a time, with only a very slight connection (figs. 5, 6, 10). The connection finally breaks and the individuals separate before the new halves are at all symmetrical with the old ones (figs. 8, 12). The granular material which formed a band across the cell now makes a thin cap for each new half (fig. 6), the material in it being continuous with the plasma membrane and forming a conspicuous example of MOLL'S embryonic substance. The change of form of this new end, resulting in an organism with symmetrical chromatophores, and the formation of the end vacuole in the granular portion follow slowly (figs. 8, 11, 12, 13). The process as we can see it, while superficially an apparently very simple one, is extremely difficult of explanation in terms of physics

and chemistry. It would seem to be necessary to assume some sense of form in the organism which MOLL has named "morphaesthaesia," a striving of the individual to attain a certain shape characteristic for the species.

The apparently unequal division of the chromatophore attracted my attention, and in order to determine just what were the proportions of the dividing parts, I made careful camera lucida drawings of six individuals of *C. Ehrenbergii* in early stages of division. It will be seen from the text figure, which is a composite one made from these six sketches, that the chromatophore division results in a cone and a frustum, and that apparently the cone is much the larger. The cubic contents of these two solids can be approximately determined, however, and the rather surprising fact is revealed that the cone has certainly not more than two-thirds the cubic contents of the frustum. It must be remembered, however, that the frustum end includes half of the large nucleus and of the vacuoles on either side of it which do not appear in the cone end. This blunt end is also the one which must undergo reconstruction, a process which undoubtedly uses up quite an amount of material such as starch, of which this portion contains more than the cone. However, it is plain that it would be improper to speak of this new half as "growing out," in the sense that it has to grow in order to become as large as the pointed end. It contains at division as much, or probably more, material than the old end; there is a reshaping of this material, but both ends take part in the growth that is to produce again a normal size in the individual.



The nucleus in these two species seems to arrive regularly at the center of the new organism before the chromatophore has finished its division (figs. 5, 6, 8, 11). This confirms FISCHER'S (**14**) observation that the nucleus in *C. moniliferum* usually arrives at the constriction in the chromatophore before it is complete, and that the separation of the last strands is apparently finished under its direction.

The resting nucleus

As commonly figured in textbooks on the algae, such as OLTMANN'S, the nucleus of *Closterium* is a lens-shaped or cylindrical body lying between the two halves of the chromatophore at the middle of the plant and containing a granular mass at its center. *C. moniliferum* is the species most frequently figured, and in it the drawings frequently show the two halves of the chromatophore pulled back from each other, with the nucleus lying as a cylindrical structure, somewhat contracted at the middle, connecting them.

I have already given a partial account (**27**) of the interesting and suggestive structure of the resting nucleus, but in connection with the present work I have of course carefully examined again great numbers of nuclei, both in whole mounts and in sections.

The nucleus is very large for an alga, and presents almost as favorable an object for study as does that body in the majority of the flowering plants. There is quite a little difference in its structure in the two species studied, although in a general way they are very similar, and are both essentially like that of *Spirogyra*. The nucleus of *C. Ehrenbergii* is in the form of a biconvex lens (figs. 14, 15), and I have never found it losing this shape as a result of the two halves of the chromatophore pulling away from each other. In end view it is circular in outline, as shown in fig. 16.

The mass of the nucleus is made up of a very fine reticulum which stains very faintly both in the triple stain and in the iron-hematoxylin, and shows a few such chromatin granules as occur on the linin threads of many of the higher plants. If such granules are present, they must be very minute or do not retain the stain. There are darker staining places in the reticulum, but I should be inclined to regard these as net-knots rather than as definite bodies. At the center of this fine reticulum is a mass of substance

which stains intensely with the safranin in the triple stain. This body, or mass of bodies, seems to be composed of globules partly fused together, and has the position and presumably the function of the nucleolus in other plant cells. In this species of *Closterium* these bodies form an irregular accumulation at the center of the reticulum, usually, at least partially, fused together in various ways, sometimes in the form of a string of beads stretched across the long axis of the cell, while at the other times the chain may be coiled around on itself, so that it simulates a spireme or has the appearance of the chromatin in the cells of the salivary gland of the *Chironomus* larvae. As would naturally result from the partial fusion of spherical granules, the individual pieces are angular on some of their sides, while rounded on others. It is not possible to determine whether, in the living cells, these globules are separate or not, or whether the partial fusion is due to fixation, such as sometimes happens in the case of chromosomes. Owing to the very faint stain which the reticulum takes and the very small size of its meshes, it is not possible to make out with any degree of certainty its relation to the central bodies.

In *C. moniliferum* (figs. 17, 18) the nucleus is composed, as in *C. Ehrenbergii*, of a very delicate reticulum containing apparently very little chromatin in the form of granules. At the center of this reticulum is usually found a large, more or less angular body, which apparently represents the irregular mass found in *C. Ehrenbergii* and the nucleolus of other plants. This nucleolus, while not having the smooth, homogeneous, globular appearance of that body in *Spirogyra*, still does not have the irregularly diffuse structure it presents in the other species of *Closterium* which I have studied. The pieces, while fused together, do not seem to form a compact mass. It has an irregular outline and shows lighter and darker places, if not stained too densely (fig. 17). The condition here would seem to represent an intermediate condition in the fusion of the nucleoli between that of *Spirogyra* and that of *C. Ehrenbergii*.

Prophase

As the formation of the spireme apparently requires a long time for its completion, the prophase is one of the stages that can be

found in great numbers. This is especially important for the question here involved, as it is one of the critical stages in which the relation of the compound nucleolus to the spireme and the chromosomes should appear.

As with mitosis in all plants, it is difficult to determine just the point at which the spireme starts to be differentiated out of the nuclear reticulum which gives rise to it. The reticulum seems to become drawn out into strands (fig. 19), but the exact method by which this takes place is not clear, for, as previously noted, the meshwork is a very delicate one, which stains very faintly and in which the structures are all very minute. These first strands, like the reticulum from which they are derived, apparently contain little chromatin in the form of granules (fig. 17), although a few such bodies are scattered in it; as the spireme grows more definite, however, there appear in it numerous small bodies which stain more densely (fig. 20).

The compound nucleolus, in the meantime, retains its position at the center of the nucleus, but the partly fused masses that composed it now becomes separated into small spheres that are independent of each other (figs. 19–23). This would seem to indicate that during the process of spireme formation, at least a portion of this central body is used. This body is suspended in the nuclear reticulum in some manner, but further than that it is not possible to see any connection between the two, such as has been shown for nucleoli in some animal cells, and by WAGER (**45**) in the nuclei of the root tip of *Phaseolus*, in which he states that the nucleolus is really only a very large granule or sphere on the linin thread. In the case of *Closterium* this central compound nucleolus apparently lies entirely clear of the reticulum, but this is undoubtedly only apparent, as it would not retain its central position in the nucleolus if it were not attached. If part of the material forming this body does pass out to assist in forming the spireme thread, the passage must occur while it is in a liquid state or as very small spheres. There is of course the possibility that the small, darker staining bodies on the spireme thread (fig. 20) may have migrated out in this manner, but the point to be particularly emphasized is that there is no passing out bodily of large pieces of this compound

nucleolus to form chromosomes on the spireme, as the compound appearance of that body in *C. Ehrenbergii* especially would indicate might happen. There seems to be no question that this body loses part of its material during the time of spireme formation, as its diminished size clearly shows, but it is no more possible here than it is in the flowering plants to prove directly that it is used to form the spireme. The spindle fibers are being formed at either pole, and it is entirely possible, as STRASBURGER believes, that it may be utilized in their formation. The apparently small quantity of chromatin in the reticulum, combined with the small size of the chromatin granules observed on it as compared with the density and size of the spireme, would certainly lead one to suspect, however, that the material from the diminishing nucleolus was being transferred to it. Of one fact there can be no question, and that is that the substratum of the spireme itself arises in the extranucleolar part of the nucleus, and not, as MITZEKEWITCH, BERGH, KARSTEN, and others have found in *Spirogyra*, inside the nucleolus, and that while the relation of the nucleolar material to this structure may not be clear, it does not seem to differ essentially from that which is found in the higher plants.

The spireme cuts transversely into chromosomes, no previous longitudinal split having been found, although it probably occurs. At the time this segmentation takes place, the spireme thread still has an irregular outline, due to small projections from its surface. These projections appear (fig. 24) also on the chromosome at first, but later disappear, and the chromosomes become long rods with a smooth surface.

In the meantime the ellipsoidal nucleus is undergoing changes in shape. Its smooth outline is lost and the nuclear walls are drawn out in places (figs. 19–21). The walls (fig. 24) are very much thickened at the future poles of the spindle, and a dense layer of fibers lies outside of them, while the equatorial walls are thin and irregular. Some of the figures obtained at this time resemble somewhat those of HERTWIG (**24**) for *Actinosphaerium*, although the thickening in the present case is not so great as is shown in his end plates. Some of these fibers extend out and are apparently attached farther back in the chromatophore, evidently serving

to attach the broad ends of the spindle, when it is fully formed and functioning, as a sort of anchorage for it. As a result of my observations I am of the opinion that the spindle itself is largely of cytoplasmic origin. The actual ingrowth of fibers which take hold of the chromosomes and later pull them apart was not observed. Strands of a lighter staining substance, probably what has been considered linin, connect the chromosomes to each other and to the nuclear wall (fig. 24). All that would seem to be necessary to form the attachment to the chromosomes would be a strengthening of those fibers extending to the nuclear wall. If this be true, there should be no fibers growing into the nucleus from the outside to take hold of the chromosomes, but only a thickening of those already present.

Metaphase

The spindle (fig. 25) is broad and as wide at the poles as at its middle. No bodies in the nature of centrosomes occur at the poles, which are broad and platelike structures (fig. 25) very similar to those described for *Spirogyra*. There are occasionally spherical bodies found in this region (figs. 28, 29), which may be metaplasmic particles, or perhaps the remains of the old nucleolus, although this point was not worked out. The spindle is attached by fibers extending from it, especially at the two sides. It is not possible to determine just where the spindle ends (figs. 25, 26). In the equatorial plate are arranged the rodlike chromosomes (fig. 25). Attached to each chromosome are fibers from either pole which pull it into two parts by a longitudinal split (fig. 26). These fibers are in the figure attached toward the middle of the chromosome, although many were also observed in which the attachment was toward their ends.

Telophase

After the chromosomes have been pulled back to the poles (figs. 27, 28) in the usual manner, they lie there in the form of a plate which is more or less crescent-shaped in cross-section (figs. 29, 30). The broad central spindle remains connecting the two poles, but gradually disappears, taking no part in the formation of the new cell wall (fig. 30). The chromosomes at either pole

apparently unite end-to-end to form a dispireme (figs. 31, 32). The nucleus with the included spireme has originally the shape of the group of chromosomes in telophase, that is, a plate, and this is retained during the reconstruction stages. The spireme stains very densely at first (figs. 29–31) while it still retains all the chromatin, but later becomes so faint that it is difficult to see (fig. 32). The formation of the reticulum from the spireme cannot be followed with any great clearness on account of the small size of the parts. There is a reticulum formed (fig. 32) in some manner, however, and on it appear bodies which stain more densely than the meshwork by which they are surrounded. At first these bodies are small and numerous, but in the later stages (fig. 33) they have fused into masses of considerable size; these masses will later form the compound nucleolus. When the nucleus has been entirely reconstructed, these bodies still lie more or less scattered, and while the nucleus is making its way back to its new position (fig. 34) there are still usually at least two groups. Apparently it is only after it has come to rest at the middle of the daughter cell that all of these nucleolar bodies take their position at the center of the nucleus. The process, as will be seen from fig. 35, is essentially the same in *C. moniliferum* as in *C. Ehrenbergii* from which the other figures were drawn. In *C. Ehrenbergii* there is only a slight fusion in places, while in *C. moniliferum* it is sufficiently complete to make a fairly homogeneous structure.

After the nuclei have been reconstructed, they begin to move out to the surface of the cell (fig. 34) and then around the chromatophore. The chromatophore being evidently a very dense structure, it is apparently much easier to go around than to penetrate it. In *Spirogyra* the central part of the cell is almost entirely free from cytoplasm, and the daughter nuclei would meet no such obstruction. As previously noted, this migration occurs along the grooves of the chromatophore (fig. 7), where, as has been shown in my paper (**27**) on the chromatophore, there are fewer strands of cytoplasm to impede its passage.

As the nucleus in both species usually arrives at the new position some time before the chromatophore has finished its division, it is very common to find figures like nos. 6, 8, 11, and 36, in which

the final separation of the two halves seems to occur "under its direction." In sections, the relation of the plasma membrane to the vacuole which is cutting the chromatophore in two is brought out very clearly (fig. 36).

It is not possible to learn more of the movement of these nuclei in sections than it is in whole mounts. The process, however, seems to be some kind of an amoeboid one, judging from the changes in shape the nuclei undergo in the process.

The formation of the daughter cell walls

There seems to be a general agreement that in the Conjugatae the new cell wall arises by a growth inward from the old wall, in which the spindle fibers, unlike those of higher plants in which a cell plate is formed, take no part. The central spindle remains in place while the wall is growing (figs. 27, 29), but seems to take no visibly active part in its formation, although there is the probability that its material may be used up to form the new plasma membrane. It is to be noted further that none of the fibers of the central spindle persist (figs. 33, 34) until they are cut in two by the ingrowth of the wall from the sides, as occurs in *Spirogyra*.

The process of new wall-formation is essentially the same in the two species of *Closterium* studied as in *Spirogyra*. After the chromosomes have been drawn back to the two poles, the large central spindle, which previously had been very conspicuous, disappears, and in the later stages of the telophase all that can be seen of it are a number of fibers extending through the region it formerly occupied (fig. 29). The cross-wall begins to grow in during metaphase, the process starting at the periphery of the cell and seeming to be due to constriction of the plasma membrane. Preceding and also accompanying this growth of the plasma membrane a layer of granular cytoplasm appears, in which the streaming movements are very noticeable in the living specimens. The new wall cuts across the spindle fairly at its center, being a third of the way across by the time the fibers have disappeared. Connecting the two nuclei is a granular band which, connecting with the ingrowing plasma membrane, gives the appearance shown in fig. 3 to the external view of the whole mount. By the time the

nuclei are entirely reconstructed, the new wall is about two-thirds of the way across in section, and the nuclei move out to the surface of the chromatophore and are making their way back to their new position before the remaining part is closed (fig. 34). It would seem from this that the presence of the two nuclei is not required for the completion of the cell division, but that the material for the new wall is there and the process is well begun before they begin their migration. It will be particularly noted that the new wall is put in at right angles to the old side walls.

It is clear that the younger end of the new individual will be covered partly by the new transverse wall that is becoming pushed out into a cone (figs. 11–13) and partly by a portion of the old parent cell wall. Where these two portions meet, the so-called girdle band appears in certain species, but in the two forms under consideration it is soon difficult to distinguish the point of union of the old and new walls, although it can be seen in individuals recently divided (fig. 8).

General considerations

FISCHER (**14**) has already pointed out the fact that it is due to the method of origin of the daughter chromatophores by division that the ridges of the chromatophore on either side of the nucleus correspond, as each ridge was cut in two when the chromatophore divided. It is to be further noted that the division of the chromatophore also explains the continuity of the outer granular layer, in which the streaming occurs, across the region separating the two halves of the chromatophore. When the chromatophore is pinched into two parts, the constriction process takes place inside this granular layer, which is not divided, but is left intact to form the outer wall of the ring-shaped vacuole. In *Closterium*, as seen during the daytime, this granular membrane is still continuous, and in it occurs the very active streaming movement between the two parts of the chromatophore.

The time relation between the division of chromatophore and nucleus in these plants is an interesting one. *Closterium* as seen in the daytime has its chromatophore divided into halves. These two halves are the result of the chromatophore division of the

preceding night. In these species of *Closterium*, however, they are further to be looked upon as being a preparation for a division of the nucleus and cell the following night, providing it has been successful in storing enough food material to make the process possible. If the individual is not able to synthesize enough starch, the nuclear and cell division is not carried out, and the two-parted chromatophore is retained indefinitely. In these species of *Closterium*, cell and nuclear division is at least a two-night process: the first night the chromatophore divides, cutting in two the greater part of the cytoplasm, but still retaining a connection between the two parts by means of the granular peripheral layer; the second night the nucleus may divide and the new wall separate the cytoplasmic halves entirely by cutting through the granular layer.

The question of the relation of the nucleolus to the chromatin, especially in the division stages of that part of the nucleus, is still an unsettled one both for plants and animals, and it is one that comes sharply into the foreground in this study of the division of the nucleus of *Closterium*. It would seem probable that, if the globular and nearly homogeneous nucleolus of *Spirogyra* contains the entire mass of chromatin in the form of a condensed spireme thread, as MOLL (**32**), MITZKEWITCH (**31**), KARSTEN (**25**), and others have found, this much more strikingly compound nucleolus of *Closterium*, arranged occasionally so as to resemble a spireme, should represent the chromosomes aggregated into a central clump. That the chromosomes are really formed from the nuclear reticulum in *Closterium* is certainly very decisive as to their morphological independence. The spireme formed outside the compound nucleolus seems indeed to be poor in chromatin, and the probability of the nucleolar material being used in the formation of the spireme cannot be denied. This, however, is also in some degree the case in the nuclei of the higher plants, and we are bound to conclude that the relations of nucleolus and chromosomes are probably the same in all nuclei, in spite of the much discussed evidence for a chromatin nucleolus in the Conjugatae.

If the spireme really thickens by the absorption of liquid material derived from the nucleolus, this can hardly be regarded,

as WAGER (45) holds, as evidence for the idioplasmic nature of the latter. The growth of the chromosomes from one cell generation to the next is still too obscure a subject to permit the use of such an interpretation.

As noted above, STRASBURGER (40), as a result of his work on *Marsilia*, in which he found the nuclear reticulum very poor in chromatin while the nucleolus was very large, holds the view that the linin may be the bearer of hereditary qualities, while the chromatin is only a food substance which divides simultaneously. This hypothesis is of interest, and it may be admitted that the recent attempts of BOVERI (4), ROSENBERG (36), OVERTON (34), and others to show that the chromosomes are permanent organs of the cell may be interpreted in support of the theory that the linin substratum of the chromosome is its more essential constituent, while the visible and more conspicuous chromatin granules only serve as conveniently scattered food.

Of interest also in this connection is the recent attempt of GRÉGOIRE (20) to show that the nuclear reticulum is all one substance, with no differentiation into chromatin and linin. These two species of *Closterium*, and in fact the Conjugatae in general, might be regarded as having a nuclear reticulum composed of a single substance; still they are perhaps not the most favorable material on which to study this question.

The chromosomes do not come bodily out of the nucleolus of *Closterium*, but that structure disappears during the prophase as in other cells, and we have as yet only theories as to its fate. In the present case the theory based on the observations of MOLL (32), MITZKEWITCH (31), KARSTEN (25), BERGH (2), and others, that as in *Spirogyra* the chromosomes are included morphologically complete in the nucleolus, would not be of service in these species of *Closterium*. In like manner the observations of VAN WISSELINGH (43) on *Spirogyra* and of Miss MERRIMAN (29) on *Zygnema*, that a number of the chromosomes come out of the nucleolus, would not apply in the present case. While the evidence in *Closterium* is not at all complete, the conclusion reached by ESCOYEZ (12, 13) on *Zygnema* and *Stypocaulon* would seem to be most in line with my own observations. These are, that while

the chromosomes do not come morphologically from the nucleolus, there is a possibility that part of the material from that body goes to form them.

The relationships of the desmids to the other groups of the Conjugatae is a rather puzzling one. While the great majority of them are unicellular, the tendency to form filaments appears in such genera as *Cosmarium*, *Euastrum*, and *Staurastrum*. BESSEY (**3**) has gone so far as to divide the desmids on this basis into three tribes: Desmideae, cells in unbranched filaments: Anthrodieae, cells solitary, elongated, but at not all or only moderately constricted; Cosmarieae, cells solitary, broad, and deeply constricted.

WEST (**46**) in studying variation in desmids came to the conclusion that the group is a degenerate one derived from a filamentous conjugate ancestor, probably among the Zygnemaceae. On this basis of degeneration he claims to be able to explain many facts previously difficult of interpretation. He holds that this degeneration has developed the highly specialized morphological characters of the different groups, thus explaining their beauty and variety of form, and that with it too has gone hand in hand the loss of sexual differentiation of the conjugating cells. LÜTKEMÜLLER (**26**), as a result of his very careful study of the cell wall of members of the different groups of desmids, has arrived at practically identical conclusions as to their phylogeny.

The position of the young transverse wall in *Closterium* also seems to throw some light on this question of phylogeny. The new cross-wall is put in at right angles to the old walls in a manner that is not in any essential different from that of the filamentous Conjugatae such as *Spirogyra*. It is only as the cells separate and the pressure is relieved on one side of this wall that the shape changes. If the cells should not separate, a filament being formed, each cell of the filament would not be essentially different from a cell of *Zygnema* with its nucleus at the middle and a half of the symmetrical chromatophore on either side. While the pointed shape which the new end assumes is obviously a secondary and acquired character, in the ontogeny of the transverse wall, we would seem to have a bit of the phylogeny of *Closterium* repeated.

Summary

1. *Closterium* divides from 10 P.M. to 5 A.M., and the new half has become practically symmetrical with the old one by 9 A.M.

2. Division is dependent upon the storage of a considerable quantity of starch in the chromatophore and around the pyrenoids.

3. The chromatophore divides by a constriction located about a third of the distance out from the middle. This constriction is due to the enlargement of a ring-shaped vacuole under the plasma membrane.

4. The resting nucleus of *C. Ehrenbergii* is made up of a very fine reticulum carrying little if any chromatin in the form of granules. At the center of this reticulum is a large compound nucleolus made up of a number of partially fused nucleoli. The resting nucleus of *C. moniliferum* has essentially the same structure, but the nucleoli at the center are more completely fused.

5. The spireme is formed outside the nucleolus and apparently separate from it. During its formation that body breaks down, but it is impossible to decide whether its material goes to the spireme or is used up for some other purpose. No chromosomes come bodily out of the nucleolus as has been described for *Spirogyra*.

6. The spindle is cylindrical, with broad poles, much resembling that of *Spirogyra*.

7. In the telophase a dispireme is formed, and in this the nucleoli reappear as small spheres which later partially fuse to form larger masses.

8. The two daughter nuclei move around the chromatophore, between its ridges, apparently in an amoeboid manner, to their new positions.

9. The new end wall is put across in essentially the same manner as in *Spirogyra*, that is, by a growth inward from the periphery.

10. Division in these species of *Closterium* is at least a two-night process: the chromatophore divides the first night; the nucleus the second night.

11. The position of the young transverse wall would seem to indicate that the pointed ends are secondarily formed, and that *Closterium* was originally a filamentous alga, which has developed the habit of breaking up into single cells.

AGRICULTURAL EXPERIMENT STATION
BURLINGTON, VT.

LITERATURE CITED

1. BEER, R., On the development of the spores of *Riccia glauca*. Annals of Botany **20**:275–291. 1906.
2. BERGHS, J., Le noyau et la cinèse chez le *Spirogyra*. La Cellule **23**:53–86. 1906.
3. BESSEY, C. E., The modern conception of the structure and classification of desmids. Trans. Am. Micr. Soc. **22**:89–96. 1901.
4. BOVERI, TH., Ergebnisse über die Konstitution der chromatischen Substanz des Zellkerns. Jena. 1904.
5. BRAUN, A., The phenomenon of rejuvenescence in nature. Roy. Soc. Trans. London. 1853.
6. CARNOY, J. B., La biologie cellulaire. Lierre. 1884.
7. DEGAGNY, C., Recherches sur la division du noyau cellulaire chez les végétaux. Bull. Soc. Bot. France **41**:588–596. 1894; **42**:319–326. 1895; **43**:12–21. 1896.
8. ———, Sur la division cellulaire chez le *Spirogyra orthospira* et sur la réintégration des matières refoulées aux pôles du fuseau. Compt. Rend. **111**:282. 1890.
9. DEBARY, A., Untersuchungen über die Familie der Conjugaten. Leipzig. 1858.
10. DIXON, H. H., The possible function of the nucleolus in heredity. Annals of Botany **13**:269–278. 1899.
11. EHRENBERG, G., Die Infusionsthierchen als vollkommene Organismen. 1836.
12. ESCOYEZ, E., La noyau et la caryocinèse chez le *Zygnema*. La Cellule **24**:353–366. 1907.
13. ———, Caryocinèse, centrosome, et kinoplasme dans le *Stypocaulon scoparium*. La Cellule **25**:179–204. 1908.
14. FISCHER, A., Ueber die Zelltheilung der Closterien. Bot. Zeit. **41**:223, 240, 256, 272. 1883.
15. ———, Ueber das Vorkommen von Gipskristallen bei den Desmidiaceen. Jahrb. Wiss. Bot. **14**:133. 1883.
16. FLEMMING, W., Zellsubstanz, Kern und Zelltheilung. Leipzig. 1882.
17. FOCKE, Phys. Stud. I. Ber. K. S. Gesell. Wiss. Leipzig. 1857.
18. GAY, FR., Essai d'une monographie locale des Conjugées. Rev. Sc. Nat. III. **3**:187–228, 285–335. 1884.
19. GOLENKIN, M., Ueber die Befruchtung bei *Sphaeroplea annulina* und über die Structur der Zellkerne bei einigen grünen Algen. Bull. Soc. Imp. Nat. Moscow 1900:343–361.
20. GRÉGOIRE, V., Les fondements cytologiques des théories courantes sur l'hérédité mendélienne. Ann. Soc. Roy. Zool. et Malac. de Belgique **42**:267–320. 1907.
21. HARPER, R. A., Cell-division in sporangia and asci. Annals of Botany **13**:467–525. 1899.

22. R. A. HARPER, Cell and nuclear division in *Fuligo varians*. BOT. GAZETTE **30**:217–251. 1900.
23. HAUPTFLEISCH, P., Zellmembran und Hüllgallerte der Desmidiaceen. Inaug. Diss. Greifswald. 1888.
24. HERTWIG, R., Ueber Kerntheilung, Richtungskörperbildung, und Befruchtung von *Actinosphaerium*. Abh. Kgl. Bayr. Ak. Wiss. II. **19**^{3}: 1898.
25. KARSTEN, G., Die Entwicklung der Zygoten von *Spirogyra jugalis* Ktzg. Flora **99**:1–11. 1908.
26. LÜTKEMÜLLER, S., Die Zellmembran der Desmidiaceen. Beitr. Biol. Pflanz. **8**:347–414. 1902.
27. LUTMAN, B. F., The cell structure of *Closterium Ehrenbergii* and *Closterium monilifera*. BOT. GAZETTE **49**:241–253. 1910.
28. MACFARLANE, J. M., The structure and division of the vegetable cell. Trans. Bot. Soc. Edinburgh **14**:191. 1881.
29. MERRIMAN, MABEL L., Nuclear division in *Zygnema*. BOT. GAZETTE **41**:43–52. 1906.
30. MEUNIER, A., Le nucléole des *Spirogyra*. La Cellule **111**: 1887.
31. MITZKEWITSCH, L., Ueber die Kerntheilung bei *Spirogyra*. Flora **85**: 81–124. 1898.
32. MOLL, J. W., Observations on karyokinesis in *Spirogyra*. Verhand. Kon. Akad. Wet. Amsterdam **9**:36. 1893.
33. NÄGELI, C., Gattungen einzelliger Algen. Zurich. 1849.
34. OVERTON, J. B., On the organization of the nuclei in the pollen mother cells of certain plants with especial reference to the permanence of the chromosomes. Annals of Botany **23**:19–61. 1909.
35. RALFS, The British Desmideae. London. 1848.
36. ROSENBERG, O., Ueber die Individualität der Chromosomen im Pflanzenreich. Flora **93**:251. 1904.
37. STRASBURGER, E., Ueber den Theilungvorgang der Zellkerne und das Verhältniss der Kerntheilung zur Zelltheilung. Bonn. 1882.
38. ———, Die Controversen der indirecten Kerntheilung. Arch. f. Mik. Anat. **23**:62. 1884.
39. ———, Ueber Zellbildung und Zelltheilung. Jena (3d ed.). 1888.
40. ———, Apogamie bei *Marsilia*. Flora **97**:123–191. 1907.
41. TANGL, E., Ueber die Theilung der Kerne in Spirogyrazellen. Sitz. K. Akad. Wiss. **85**^{1}:268. 1882.
42. VAN WISSELINGH, C., Ueber den Nucleolus von *Spirogyra*. Bot. Zeit. **56**:195–226. 1898.
43. ———, Ueber Kerntheilung bei *Spirogyra*. Flora **87**:355. 1900.
44. ———, Untersuchungen über *Spirogyra*. Bot. Zeit. **60**:115–138. 1902.
45. WAGER, H., The nucleolus and nuclear division in the root apex of *Phaseolus*. Annals of Botany **18**:29–55. 1904.

46. WEST, G. S., On variation in the Desmideae and its bearing on their classification. Jour. Linn. Soc. Bot. **34**:376. 1899.
47. WOLFE, J. J., Cytological studies on *Nemalion*. Annals of Botany **18**: 607–630. 1904.
48. ZACHARIAS, E., Ueber den Nucleolus. Bot. Zeit. **43**:257–265, 273–283, 289–296. 1885.

EXPLANATION OF PLATES XXII AND XXIII

All figures were drawn with the aid of a camera lucida; the majority of them with the Leitz $\frac{1}{16}$ achromatic objectives and eyepieces, and the others with the Zeiss 3 mm. apochromatics and compensating oculars. The approximate magnification is indicated after each figure. Figs. 1–13 are from whole mounts in glycerin, and are diagrammatic in that they do not show the slight shrinkage of cell contents due to fixation and transfer to glycerin. The other figures are all from sections 5–10 μ in thickness, stained with the triple stain, except fig. 29, which is from a slide stained with iron-hematoxylin.

C. Ehrenbergii

FIG. 1.—Chromatophore just beginning to show constrictions; ×300.

FIG. 2.—Nucleus in metaphase; transverse cell wall beginning; ×300.

FIG. 3.—Transverse cell wall nearly complete; nuclei not showing; ×300.

FIG. 4.—Transverse wall complete; nuclei moving back to their new position; ×300.

FIG. 5.—New ends beginning to round off; nuclei at constriction; ×300.

FIG. 6.—New end still further rounded; ×300.

FIG. 7.—Position of nucleus between the ridges of the chromatophore as seen from the convex side; ×300.

FIG. 8.—Individual with asymmetrical halves; the chromatophore not yet completely divided; the new tip beginning to assume its pointed shape; ×300.

C. moniliferum

FIG. 9.—First appearance of constriction in the chromatophore; pyrenoids dividing; ×550.

FIG. 10.—Individual with two halves just ready to separate; ×550.

FIGS. 11–13.—Various stages in the change of form of the new halves; fig. 11 showing pyrenoids dividing; fig. 11, ×550; figs. 12, 13, ×475.

C. Ehrenbergii

FIGS. 14, 15.—The resting nucleus in side view; ×1800.

FIG. 16.—Resting nucleus in end view; ×1800.

C. moniliferum

FIG. 17.—Resting nucleus in side view; ×1800.

FIG. 18.—Resting nucleus in end view; ×1800.

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FIG. 19.—First traces of spireme formation; ×1800.

FIGS. 20, 21.—Later stages of spireme; ×1800.

FIG. 22.—Detail spireme showing chromatin granules; ×1800.

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FIG. 23.—Spireme; nucleolus breaking up; ×1800.

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FIG. 24.—Chromosomes formed; ×1050.

FIG. 25.—Metaphase; ×1050.

FIG. 26.—Detail of metaphase; ×2400.

FIG. 27.—Anaphase; ×1050.

FIG. 28.—Late anaphase; ×1800.

FIG. 29.—Telophase: spindle fibers still showing; ×1050.

FIG. 30.—Telophase; spindle fibers have all disappeared; wall touching remains of central spindle; ×1050.

FIGS. 31, 32.—Telophase; dispireme; nucleoli reappearing in fig. 32; ×2400.

FIG. 33.—Late telophase; nucleoli fusing to form large masses; ×1050.

FIG. 34.—Nuclei moving out to surface of chromatophore; both nuclei still showing a number of masses of nucleolar material; cell wall not yet completed; ×1050.

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FIG. 35.—Late telophase; nuclei reconstructed, but nucleoli not yet fused to form a single mass; cell wall beginning; ×1050.

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FIG. 36.—Nucleus in its new position at constriction in chromatophore, but latter not yet divided; *pp*, granular layer; ×1050.


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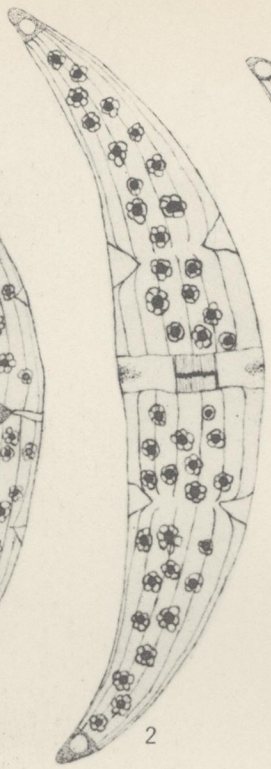
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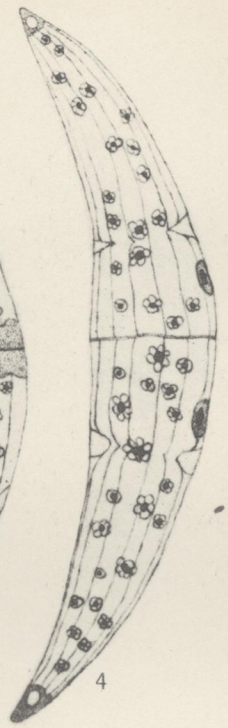
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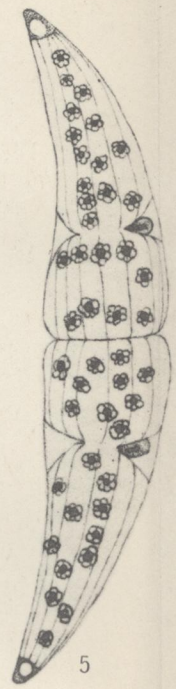
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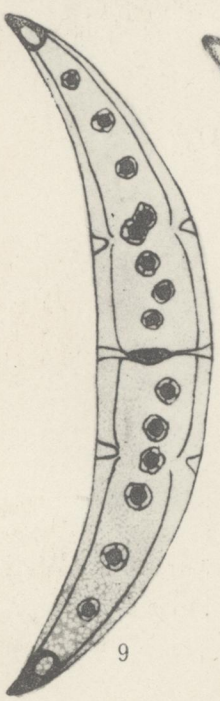
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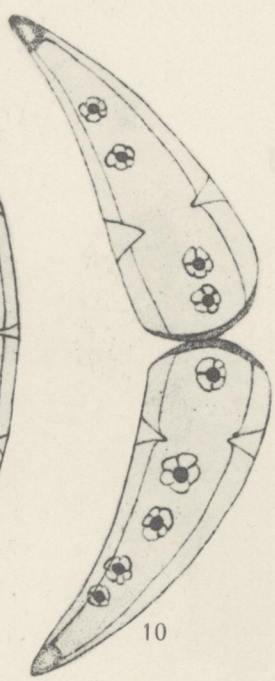
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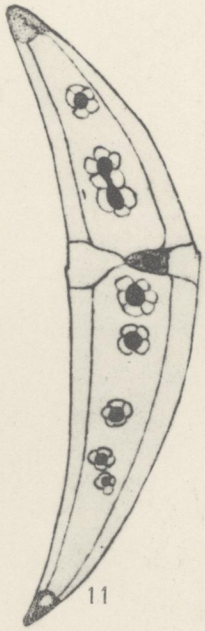
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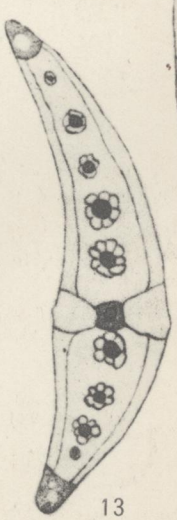
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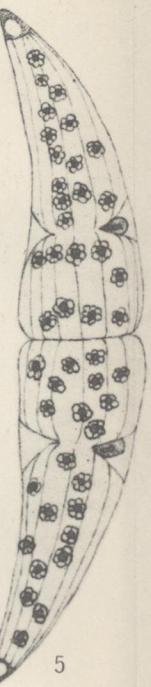
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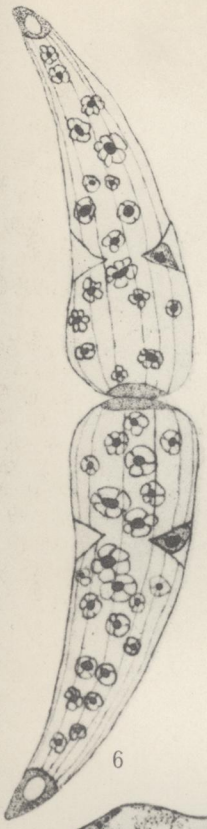
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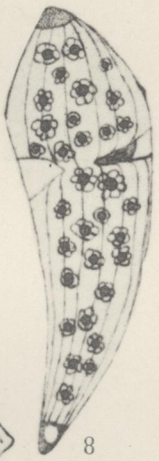
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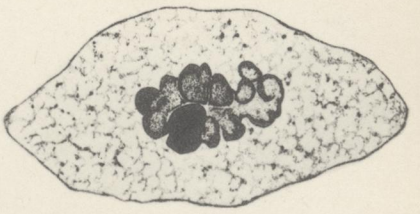
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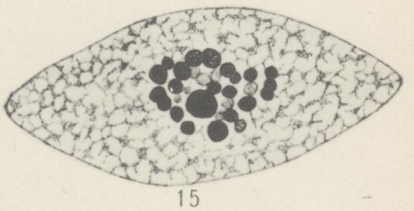
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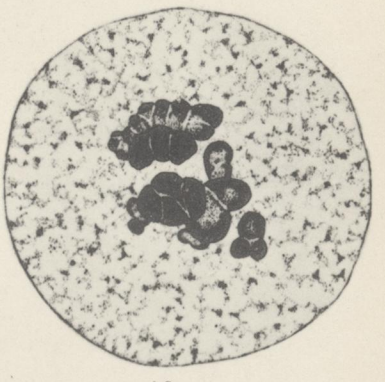
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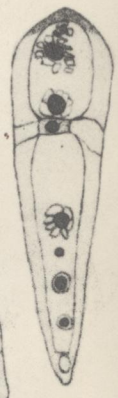
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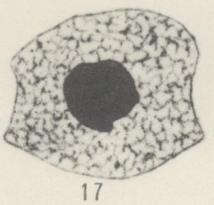
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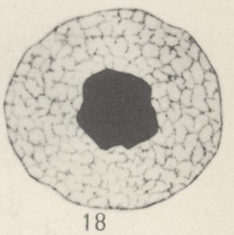
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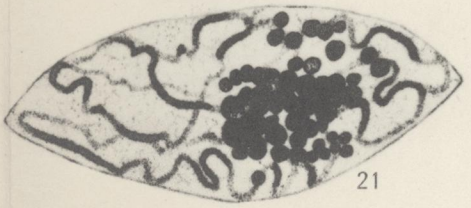
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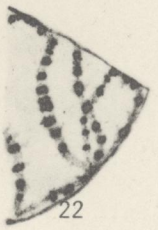
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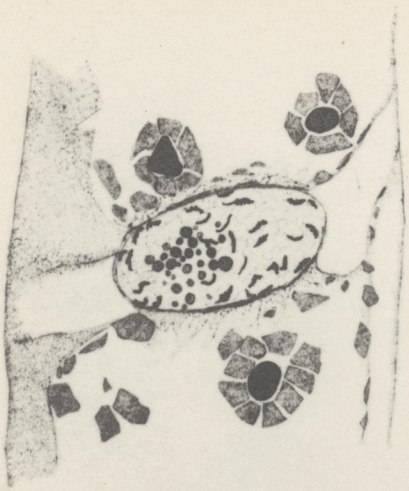

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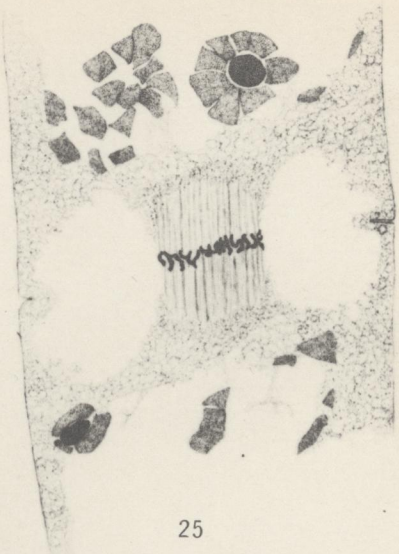

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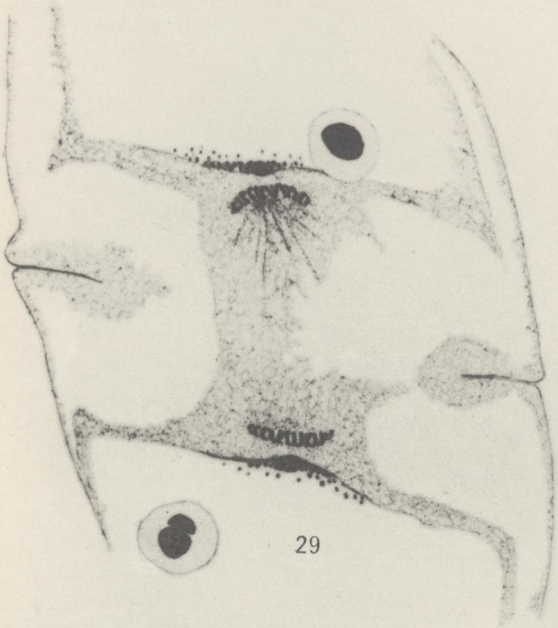

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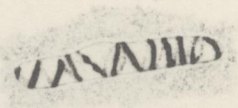

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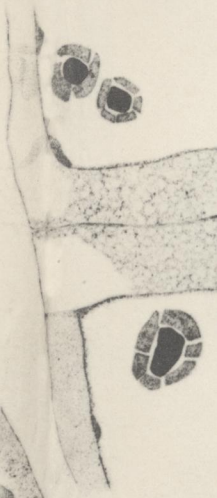

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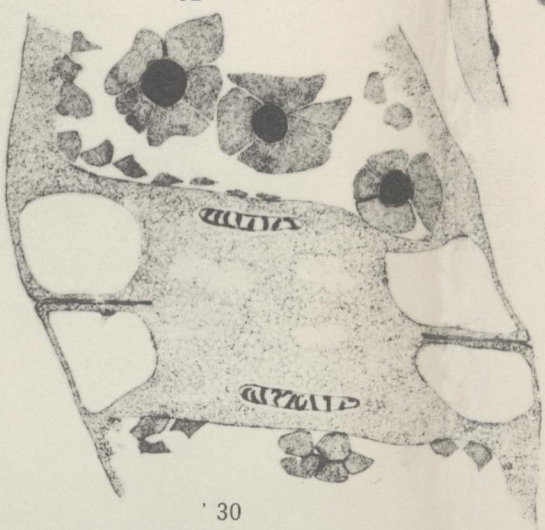

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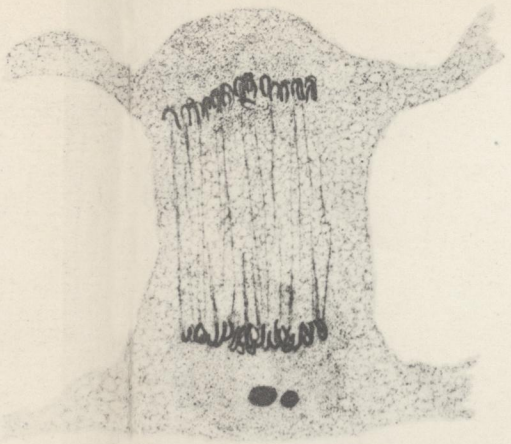

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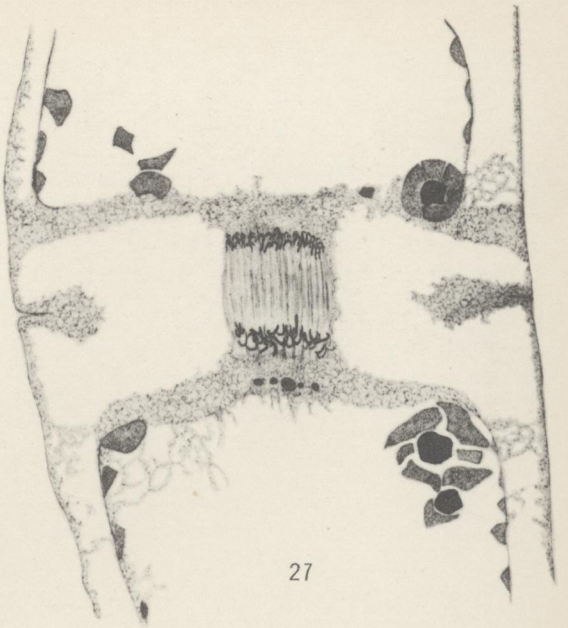

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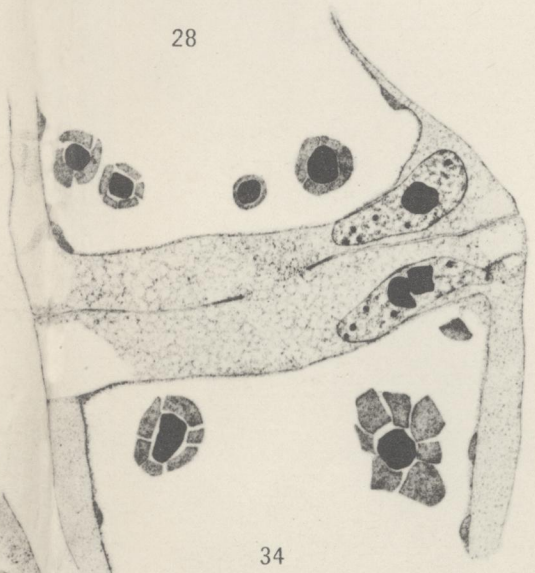

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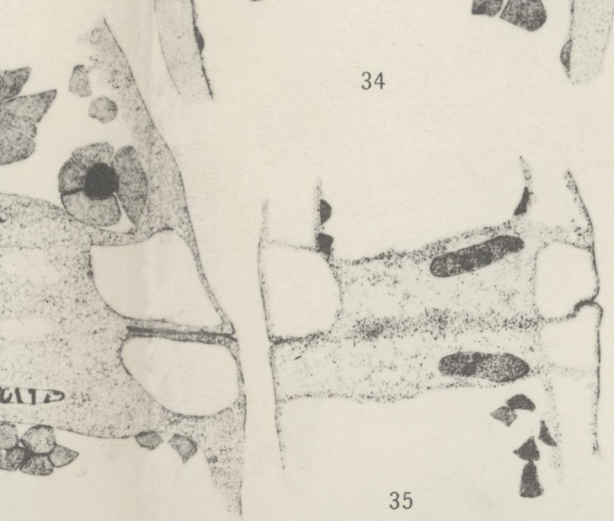

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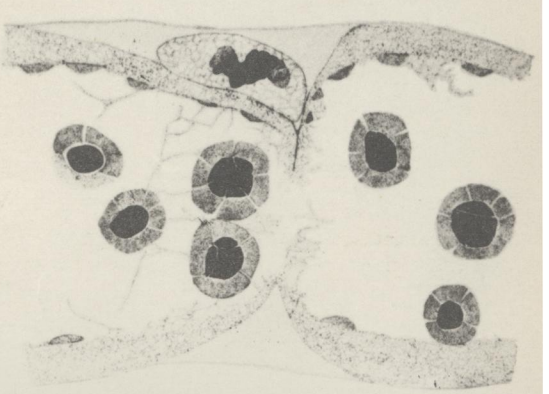

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